Detection of a DNA Sequence by Surface Enhanced Resonance Raman Scattering of a Modified DNA Probe

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INTRODUCTION

The technique we are reporting, Surface Enhanced Resonance Raman Scattering, SERRS, exploits the light scattering, or Raman scattering, produced when a light beam illuminates molecules. The effect is weak and depends on the molecular structure so that some molecules are weaker scatters than others are. Accordingly signals from strong scatters can be detected in the presence of weaker ones such as water. The effect is enhanced up to 10^6 fold if the analyte is adsorbed onto a roughened metal surface, i.e. Surface Enhanced Resonance Scattering (SERS). A further enhancement is achieved by utilizing a dye as the analyte and by tuning the laser excitation frequency to the maximum of the dye chromophore to obtain resonance scattering from the surface i.e. SERRS.

The method therefore requires the adhesion of the chromophore to a metal surface to obtain the resonance scattering. The procedure can be carried out in an aqueous environment using silver metal colloidal particles in suspension. By adsorbing the chromophore into interstices between silver particles in aggregates of the colloid the surface scattering effect can be obtained.

Work conducted at Strathclyde University over the past three years has developed the means to adsorb negatively charged DNA onto negatively charged silver particles. This can be promoted and controlled to the extent that SERRS for DNA detection has become possible.

By the use of special chemistry DNA molecules bearing a dye label have been adsorbed onto the surface of silver particles which have been aggregated under controlled conditions. This has enabled the detection of approximately one molecule of labeled DNA.

Work to date has been carried out using fluorescent dye labels however there is a wide range of potential chromophores that may be linked to DNA therefore in addition to sensitivity the method offers the potential of simultaneous detection of many differently labeled DNAs.

The very high sensitivity of the SERRS technique makes possible the detection of restriction fragments from much smaller sample sizes. This may be of significance in

cases where restriction digests are to be analysed and samples are of restricted size. The analysis of PCR fragments may also be facilitated by requiring fewer reaction cycles or even by avoiding the need for geometric amplification at all. The technique avoids the need for radioisotopes or for large conjugated molecules such as enzymes or biotin and therefore facilitate hybridisation to surface bound target sites. It is expected that a large range of highly SERRS active chromophores will become available as other aspects of the chemistry are developed in parallel work being conducted at Strathclyde University.

This paper describes the detection of dye labeled DNA by SERRS by utilizing two improvements over existing procedures; i.e. the controlled aggregation of silver colloid particles by the use of spermine and the use of a specifically designed oligonucleotide incorporating modified bases. This oligo can be directly applied to conventional molecular biology assays. These developments take SERRS much closer to competing with fluorescence in standard procedures.

METHODOLOGY AND RESULTS

A surface which has proved particularly suitable to produce strong SERRS is a silver colloid aggregated to provide regions of high electric fields in the interstices. However, when an attempt was made to use the conventional method of SERRS detection with colloid, for a sample of DNA labeled at the 5' terminus with the fluorescent dye label "HEX" to produce a resonant probe, poor scattering was observed. The problem appeared to be that the surface of the silver had a negative charge and the negatively charged DNA did not adsorb to the surface of the silver due to the electrostatic repulsion. This problem will also have adversely affected previous SERS studies which use colloid.

Two new procedures were utilised to overcome the problem. In order to achieve the maximum enhancement from the surface of the colloid, the aggregation procedure is critical. Inorganic ions (Na^+, Mg^{2+}, Cl^-) or acid are often used as aggregating agents. They appear to act by reducing the surface charge on the colloid. These methods are ineffective with DNA because aggregation is achieved without attachment of the DNA to the silver surface. In a new departure, the organic polyamine, spermine, was used. It interacts with DNA to balance the charged phosphate groups thus acting as a charge modifier of DNA. It was found to be an effective agent for controlled aggregation of the colloidal particles. A Transmission Electron Micrograph (TEM) of the aggregated colloid shows how the colloidal particles are packed closely together to provide the areas of high electric field necessary for the surface enhancement. (Fig. 1)

In a further step the DNA was chemically modified to incorporate a propargylamino modified nucleoside (Fig. 2) which was prepared according to the procedure of Cruickshank *et al*.[(1)] and incorporated into DNA by routine solid phase synthesis. By modifying several bases in the oligonucleotide, positively charged amine groups within the DNA are produced which interact strongly with the negative colloidal surface. This improved surface attraction of the DNA and permitted SERRS of the active label to be obtained. The addition of the propargylamino modified nucleosides will stabilise any duplex formed by hybridisation and it is suspected to improve base specificity. Hence the

modified oligonucleotides will not only adhere to the colloidal surface but act as very specific capture probes for target sequences.

The target signals were obtained when the DNA was mixed with an excess of spermine prior to addition to the colloidal suspension to neutralise the DNA. After addition of the DNA/spermine mixture to the colloidal suspension, the propargylamino modification attaches the DNA to the surface of the colloid. The excess spermine aggregates the colloidal particles to produce a mixture that allows SERRS signals to be accumulated (Fig.3).

By adopting this procedure we have been able to detect both single and double stranded DNA. The first sequence successfully detected was a 17mer labeled at the 5'-terminus with the commercially available dye, HEX. (Fig. 4) Four main bands were observed, 1302cm^{-1}, 1341cm^{-1}, 1502cm^{-1} and 1629cm^{-1} which correspond to symmetrical modes of in-plane C-C stretching vibrations. The 17mer contained six of the propargylamino modified bases, HEX-T*T*C GCC T*T*A GCC AAT* T*C. SERRS was obtained down to an overall concentration of 8 x 10^{-13}M, 4 x 10^{-16} moles by starting with a 1 x 10^{-11} M solution and adding 40ml to 500ml of colloid and water (1:4).

One inconsistency found with the dilution study was that the peak found at 1502cm^{-1} appeared to diminish in size as the concentration was reduced and the peak at 1543cm^{-1} increase. As the DNA is diluted the pattern of surface adsorption will alter thus producing a change in enhancement of different modes.

To provide an estimate on the sensitivity of the system the actual number of molecules examined at any one time in the interrogation volume was calculated. The interrogation volume was assumed as being a cylinder of dimensions d = 5mm and h = 20mm. This produced a volume of 4 x 10^{-16} m^3 or 4 x 10^{-10} cm^3. The lowest concentration of oligomer detected was 1 x 10^{-11} M. 40 ml of this solution was added to 500ml of colloidal suspension thus producing a final concentration of 8 x 10^{-13} M or 4 x 10^{-16} moles. Of that solution only 400ml were used which equates to 3.2 x 10^{-16} moles or by multiplying by Avogadro's constant 192.7 million molecules. By ratio of interrogation to sample volumes we can say that there are only 0.08 molecules being irradiated at any one time.

Various longer chain oligonucleotides have been synthesised to examine the effect length had on the signal. A 26mer primer was synthesised with a $(T*C)_6$ repeat at the 5'-terminus and also a HEX label, HEX-T*CT* CT*C T*CT* CT*C GTG CTG CAG GTG TAA ACT TGT ACC AG. The resulting modified 38mer oligo provided strong SERRS signals thus indicating that the HEX label was close enough to the surface to experience surface enhancement.

As the modified 5' tail was sufficient in this case it was decided to examine extension products primed from this 38mer and also a reverse primer. The reverse primer enabled the controlled synthesis of two extension products, a 120mer and a 254mer. Both extension products were purified by gel electrophoresis prior to examination and were double stranded containing six propynyl modified groups and one HEX label per duplex.

Detection of the 120mer was achieved at an estimated concentration of $4x10^{-11}$M in 1ml which translates to approximately 10 molecules under examination at any one time. A similar result was obtained for the longer extension product.

CONCLUSIONS

Previous levels of detection, of individually labeled DNA sequences, by colloidal SERRS have been surpassed by several orders of magnitude. This was made possible by examining the surface chemistry in detail and devising chemical modifications to overcome the surface adsorption problem. The experiments described above illustrate that SERRS is capable of detecting DNA without the need for an amplification step currently required for genome analysis. The process itself is reliable, reproducible, and highly sensitive. It is selective in that the sharp signals provide a "fingerprint" of the specific dye chromophores[(3)] and single components of mixtures can be readily identified thus diminishing the need for a separation step. In addition, the use of non fluorescent as well as fluorescent labels will increase the range of labels available for use with DNA. This unique approach, requiring surface binding rather than solution detection, produces opportunities to develop a range of new DNA analytical methods

REFERENCE

Cruickshank, K.A. and Stockwell, D.L.: *Tetrahedron Letts.* 1988, **29**(41):5221-5224